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Strong acid treatment for making smears of tobacco microspores [1]

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Abstract: A procedure developed for identifying the developmental stage of tobacco microspores is described. Fixation in Carnoy's solution (ethanol, chloroform, acetic acid, 6 : 3 : 1) for at least 30 min is followed by soaking in 70% ethanol. Storage, if necessary, at 5-8°C is recommended. Maceration in a 2 : 1 : 1 mixture of 15% CrO_3, 10% HNO_3, and 5% HCl for 7-10 min. is required to remove spore walls. Hardening is then done in 1 : 1 95% ethanol-propionic acid and staining in 1% aceto-carmine for 20-30 min.

The developmental stage at which anthers are excised and cultured is one of the most critical factors for androgenesis. Generally, it occurs somewhere around first pollen mitosis in tobacco (4,6,7,8). The conventional methods of fixing and staining at this stage leave the walls of the spore intact. As the spore wall of microspore always stains intensely and causes poor contrast, sometime it is difficult to ensure the exact microspore stage. Burns and Yang recommended the use of acid maceration method of handling the microspores of *Nicotiana* (3), Bernardo described the use of CrO_3 in conjunction with HNO_3 and HCl were satisfactory for the preparations of the thicker-walled *Gossypium* microspores (1). The present technique adopts these reagents for removing the spore walls.

The procedures shown below were developed according the Bhadure and Majumdar (2), Burns and Yang (3), Bernardo (1), Jagathesan and Sreenivasan (5) for identifying the development stages of tobacco microspores:

1. Fix anthers in Carnoy's solution for 30 min.
2. Transfer to 70% ethanol, store at 5—8°C if necessary.
3. Rinse with 30% ethanol for 15 min.
4. Rinse with distilled water for 30 min.
5. Hydrolyze in 1 N HCl at 60°C for 10 min.
6. Rinse in distilled water
7. Macerate in drop of water on slide.
8. Squash anther, remove anther wall and apply cover-slip.

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9. Rinse and clear tapetal cells by passing water from one side of cover slip to another with aid of blotting paper.

10. Dissolve exine by introducing freshly prepared 2 : 1 : 1 mixture of 15% CrO_3 : 10 % HNO_3 : 5% HCl under cover slip and let stand for 7—10 min. The time element of strong acid treatment is very critical. If the treating time by strong acid is too short, the spore wall will not be sufficiently removed. On the other hand, after the sporewall has disappeared the entire cell contents will disintegrate within a few minutes. For our purpose, 7—10 min. in the acid solution at room temperature (ca. 26°C) is adequate. The proper timing can be determined by observing under low magnification of a microscope. Upon the disappearance of the spore walls the slide should be rinsed with distilled water immediately.

11. Rinse slide with distilled water as in step 9.

12. After the strong acid treatment, the protoplasts are very soft. They must be hardened before the chromosomes can be stained. In tobacco microspore, the cytoplasm is hardened by displacing the distilled water with 1 : 1 mixture of 95% ethanol and propionic acid. Since hardening solution evaporates rapidly, the treatment should be continued by adding acid-alcohol at intervals of at least 3 min.

13. Stain with 1% aceto-carmine, leave in stain until cells turn red (20—30min.).

14. Place slide over alcohol lamp and heat gently.

15. Gently apply pressure onto cover slip while still hot.

16. Examine under microscope.

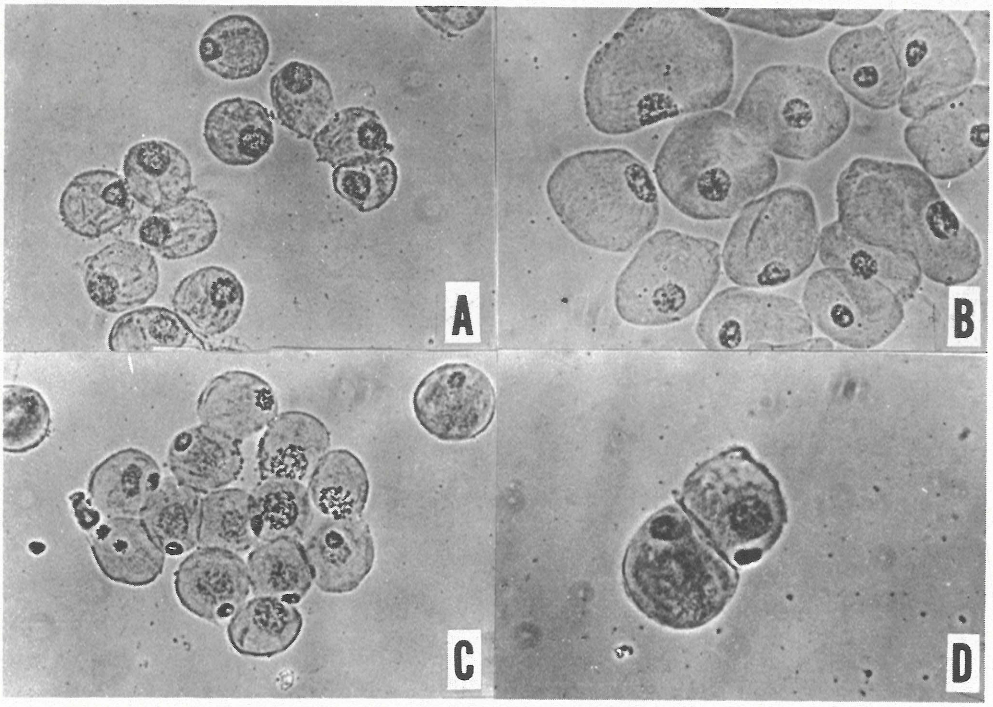

Fig. 1. The developmental stage of microspores of *N. tabacum* at early uninucleate stage (A), late uninucleate stage (B), first pollen mitosis (C), and early binucleate stage (D)by strong acid treatment (7 min,) and aceto-carmine staining preparation.

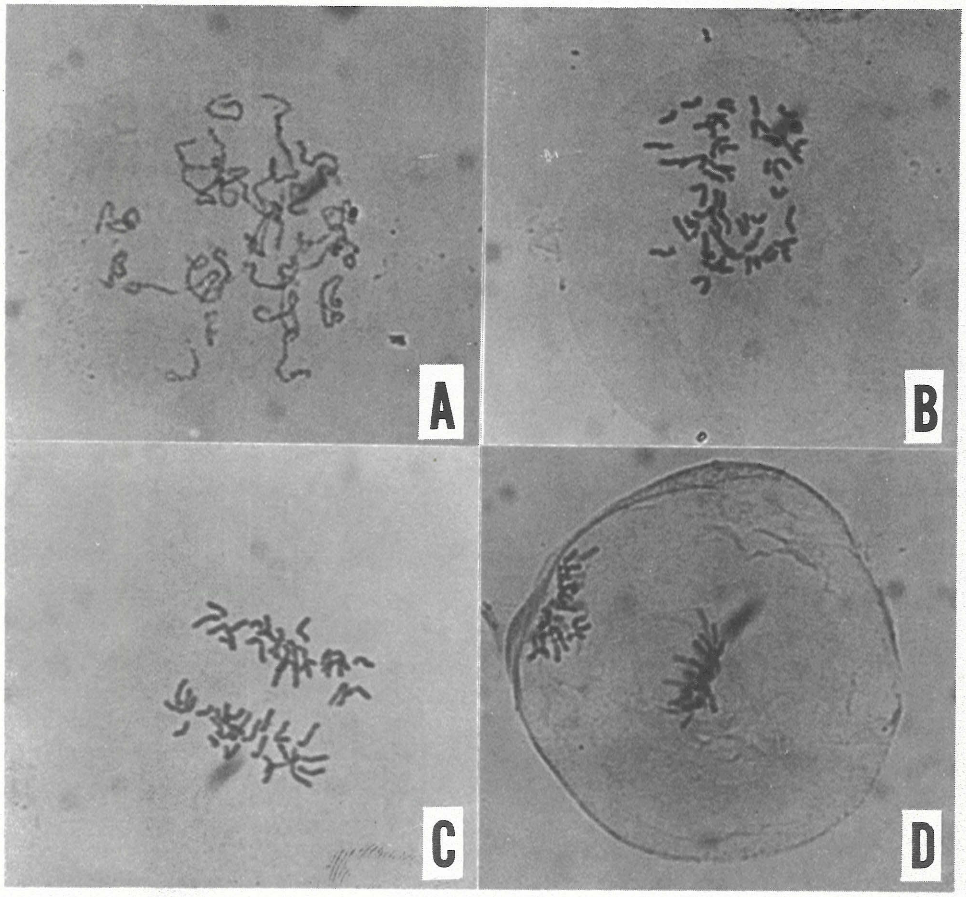

Fig. 2. The dividing pollen grain of *N. tabacum* at prophase (A), metaphase (B), anaphase (C), and telophase (D) of first pollen mitosis by strong acid treatment (10 min.) and aceto-carmine staining preparations.

Only by experiment can the technique be adjusted to produce satisfactory results in any given situation. Factors such as freshly prepared acid solution, the time needed to remove the spore wall, the time needed to wash and harden the cytoplasm and the time needed to stain may require adjusting, depending on the maturity of the spore wall.

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菸草小胞子強酸處理之醋洋紅塗抹法[1]

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摘 要

菸草小胞子不同發育時期之醋洋紅壓潰檢定法其過程如後：將菸草花葯固定於 Carnoy's 固定液中至少30分鐘，然後立即浸入70%酒精中，若需貯藏則以 5°～8°C 之溫度較爲適當，以新鮮配製 2：1：1之 15% CrO_3, 10% HNO_3 及 5% HCl 處理小胞子 7～10 分鐘以溶解細胞壁，其原生質體經 1：1之 95% 酒精及 propionic acid 硬化 3 分鐘，再以 1%醋洋紅染色 20～30 分鐘。

1.臺灣省農業試驗所 研究報告 第 919 號。

2.本所農藝系技正。臺灣省 臺中縣 霧峰鄉。